Medical Science

25(113), July, 2021

Antioxidant action of green tea extract in alloxan-induced diabetic albino rats: An experimental laboratory-based study

To Cite:
Ali AHA, Alali AY, Alhajri RA, Aldawsari AM, AlHeseni AH, Aldosari NS. Antioxidant action of green tea extract in alloxan-induced diabetic albino rats: An experimental laboratory-based study. *Medical Science*, 2021, 25(113), 1542-1550

Author Affiliation:
[1]Anatomy Department, College of Medicine, Prince Sattam Bin Abdulaziz University, Alkharj 11942, KSA
[2]Anatomy Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[3]College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA

✉**Corresponding author**
Anatomy Department, College of Medicine, Prince Sattam Bin Abdulaziz University, Alkharj 11942, KSA
Anatomy Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
Email: alihassan3750@yahoo.com / a.ali@psau.edu.sa

Peer-Review History
Received: 19 May 2021
Reviewed & Revised: 20/May/2021 to 18/June/2021
Accepted: 19 June 2021
Published: July 2021

Peer-review Method
External peer-review was done through double-blind method.

Ali Hassan A Ali[1,2]✉, **Ali Yaqoub Alali**[3], **Rasheed A Alhajri**[3], **Abdulrahman M Aldawsari**[3], **Ammar H AlHeseni**[3], **Nasser S Aldosari**[3]

ABSTRACT

Background: Green tea extract can regulate blood sugar and encourage weight loss due to its high antioxidant content. Several studies have shown that green tea extract can improve general health and may help protect against many diseases. Most patients with diabetes are associated with poor fertility. *Aim of the work*: The present study aimed to evaluate the importance of green tea extract supplementation in reducing metabolic abnormalities associated with alloxan-induced diabetes in male albino rats. *Material and Methods*: The number of male rats was thirty, weighing 100-110 grams were used in the current study. The rats were divided equally into three groups that included control, diabetic and diabetic took green tea extracts orally (50 mg/Kg/day) for three weeks. To induce diabetes one dose (120 mg/kg) of alloxan was used. Diabetic rats were given "50 mg/kg body weight green tea extracts orally for thirty days twice per day. The morphological and histological structures of the testis were compared in different groups of the rats. *Results*: It was revealed that the morphological changes of the testis observed in diabetic groups were siginficantly improved after treatment with green tea compared to the control group. *Conclusion*: These results prove that green tea improves oxidative damage caused by diabetes in the testis of male rats.

Keywords: Testis, green tea, Antioxidant, alloxan, Albino rat.

1. INTRODUCTION

Diabetes mellitus is one of the environmental factors that damages the testis and thus affects the fertility in men (Sebastian & Raghavan, 2015). Worldwide, diabetes is a common metabolic disorder. It is associated with disturbances in the metabolism of carbohydrate, fat, and protein (Moore et al., 2004). Experimentally, it was found that the induction of diabetes in male rats is accompanied by change in the performance of the genital system (Kuhn-

Velten et al., 1982; Morimoto et al., 2005). It has been proven that diabetes induction affects the functions of the testis due to insulin defiency and consequently impairment of regulation of insulin action on the Sertoli and Leydig cells (Ballester et al., 2004). In diabetic cases there are several studies that have shown changes in the structure of reproductive system (Cai et al., 2000; Sanguinetti et al., 1995; Oztürk et al., 2002).

Diabetes mellitus is a multifactorial diease with defiency in scavengers enzymes of ROS (reactive oxygen species) (Kesavulu et al., 2002). Chronic hyperglycemia is the prime cause of a number of long term diabetes complications. The free radicals main source is protein glycation caused by hyperglycemia. ROS can directly cause degeneration of cell by activation of several stress-sensitive cellular pathways, which lead to diabetes complication of (Evans et al., 2003). Several secondary metabolites of the plant showed antioxidant improved effect on damage induced oxidative stress in diabetes (Vieira et al., 2020; Ugochukwu et al., 2003). Traditional medicine practices, are responsible for an neutral responsibility in PHC (primary health care) despite the accessibility of modern medicine (Wazaify et al., 2011). Diatery antioxidants, in general, are often safe substances found in the medicinal plants and have attractive effects in the complementary medicine including an important effect in reducting oxidative stress (Hamden et al., 2009; Elzoghby et al., 2014; Hassen et al., 2021). The polyphenols and catechins that make up large groups of nutritional antioxidants are specially found in the green tea (Adel et al., 2009; Higdon & Frei, 2003). In addition to its antioxidant effect, these nutrients have anti-diabetic, antihypertensive, anti-oxidative, anti-inflammatory, and anti-fungal functions (Heikal et al., 2013; Al-Attar & Abu Zeid, 2013). Therefore, green tea consumption is associated with a lower death rate specially from the cardiovascular strokes (Kuriyama et al., 2006).

The protective mechanism of green tea consumption against chronic diseases is unclear, however, it has been suggested that green tea consumption has a protective role with these diseases may be invoked to the antioxidant action of its polyphenols and catechins (Higdon & Frei, 2003; Frei & Higdon, 2003; Lotito & Frei, 2006; Bagherpour et al., 2019).

The present study aimed to investigate the antidiabetic effects of green tea aqueous extract on testisin male albino rats with diabetes mellitus induced by alloxan.

2. MATERIAL AND METHODS

300 mg tablets green tea extract manufactured by Tecno Med Company – KSA was used in this study. The tablets were mashed then, the desired amount dissolved in distilled water. It was filtered after cooling to room temperature. Then, the extract was prepared daily. At that moment, It was stored in in a refrigerator.

Animals

In this experimental study, 30 adult, healthy, male Wistar albino rats (300-350 grams, 3 months old) were used. They were gained from an animal house of the College of Pharmacy, KSA. They were kept under controlled standard animal housing situations and moisture at the Animal Care Facility at the Department of Clinical Pharmacy (Fig.1). The pellet diet composed of 23% protein, 5% lipids, 4% crude fiber, and 55% nitrogen-free extract. The rats were kept under control for approximately 2 weeks befor the start of the experimentation to allow acclimatization. Diabetes mellitus was induced in animals except for the control group with one dose (120 mg/kg) of alloxan dissolved in saline, injected intraperitoneally (Malaisse, 1982).

Experimental design

Three experimental groups, ten rats for each, were used:

Group I: Non-diabetic control rats. Group II (was called diabetic group): in this group the rats were intraperitoneally injected with a alloxan (single dose of 120 mg/kg melted in solution of saline).

Group III (Green tea+ Diabetic group): in this group the rats were intraperitoneally injected with aalloxan (single dose of 120 mg/kg melted in solution of saline). Then, treated orally with green tea extract 50 mg/kg body wt. twice/day green tea extracts orally for 30 days.

After sacrificing animals, their testes were isolated from the scrotum. The testicular samples were taken for staining, and then examined microscopically.

The specimens were prepared for histological and histochemical studies by fixation in Carnoy's fluid and formalin solution (10% neutral buffered). Harris's haematoxylin and eosin (H & E) staining on paraffin sections was used for histological study (Bancroft & Gamble, 2008). To detect collagen fibers, Mallory's trichrome stain was used in paraffin sections. Paraffin sections of 5μm thickness were prepared and stained with Feulgen stain for histochemical study, (Bancroft & Gamble, 2008). Then, all the

stained sections were examined with light microscopy. After that, the sections were photographed and all the differences detected between the three groups were discussed.

For immunohistochemical study, the paraffin removed from testes sections with xylene. Then incubated with anti-caspase–3 (1:100; Abcam, Ab4051). After washing the slides with saline, the sections were incubated at room temperature for one hour. Next, 3-amino-9-ethylcarbazole, a chromogen was detected.

Figure 1 A) Male Wistar albino rats an animal house of the College of Pharmacy. B) Testes after excion.

Statistical analyses

Statistical analyses were performed using ANOVA test. A P value < 0.05 was considered statistically significant.

3. RESULTS

Histological results: the control group showed several seminiferous tubules cut in various planes of sections. The seminiferous tubules (S. Ts) were highly convoluted and lined germ cells in various stages of spermatogenesis and spermiogenesis which are collectively referred to as spermatogenic series. Non germ cells called Sertoli cells were well preserved. In the interstitial spaces between the tubules, endocrine cells called Leydig cells were found singly or in groups in the supporting tissue (Fig. 2). The histological examination of testicular tissue in the rats of untreated diabetic showed irregularity of the shapes of seminefrous tubules with considerable reduction in its diameters compared to the control group. In addition, the germinal epithelium showed a clear disruption of the germinal epithelium. Furthermore, the main type of cells seen were the spermatogonia cells (Fig. 3, & Table 1). Multinucleated cells were also detected in S. Ts. In addition, an amorphous substance was found in the interstitial connective tissue with subsequent widening of the interstitial spaces spaces (Figure 4). The green tea treated group showed a considerable recovery of the diameters of S.Ts compared to the diabetic group (Figure 3). It also showed a significant recovery of the interstitial spaces to normal levels with considrable excess in the collagen fibers (Figure 4).

Immuno histochemical study

The immuno histochemical examination of the control group demonstrated moderate expression of staining of Caspase-3 with a small amount of +ve cells of caspase-3 (Fig. 5 & Table 1). On the other hand, in group 2 diabetic rat, a marked significant rise in the

number of cells of caspase-3+ve was noticed in the S. Ts (Fig. 4 & Table 1). The green tea treated group showed a marked significant rise in the quantity of caspase-3+ve cells (Fig. 4 & Table 1).

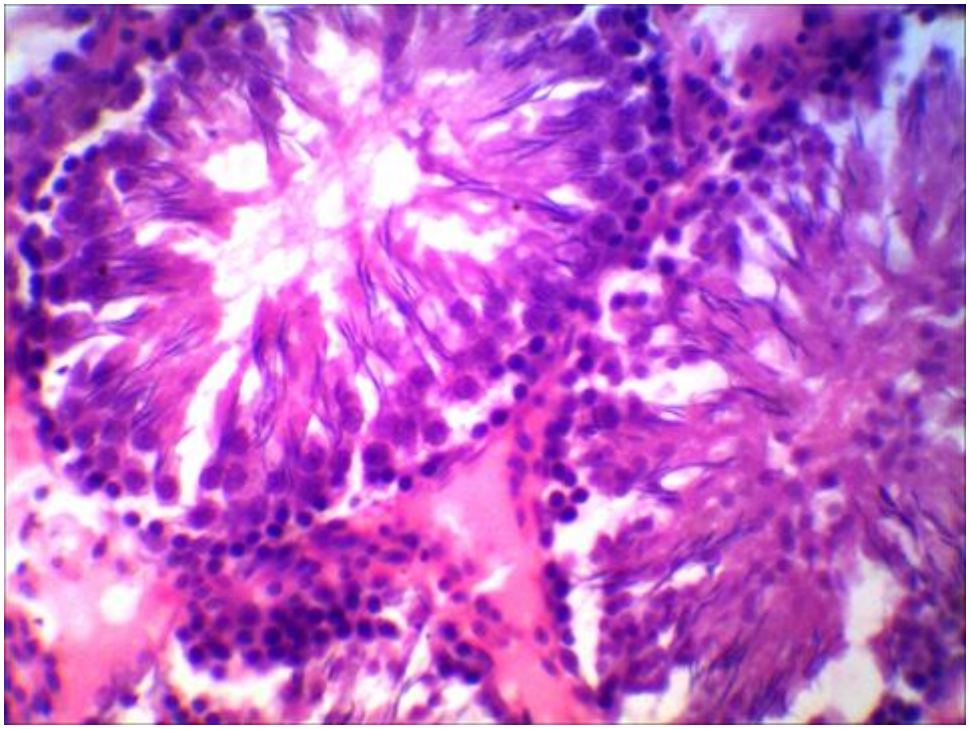

Figure 2 Histological light photomicrograph of testis sections (H and E) of control group shows normal arrangement of seminiferous tubules, sperms and normal architecture interstitials cells.

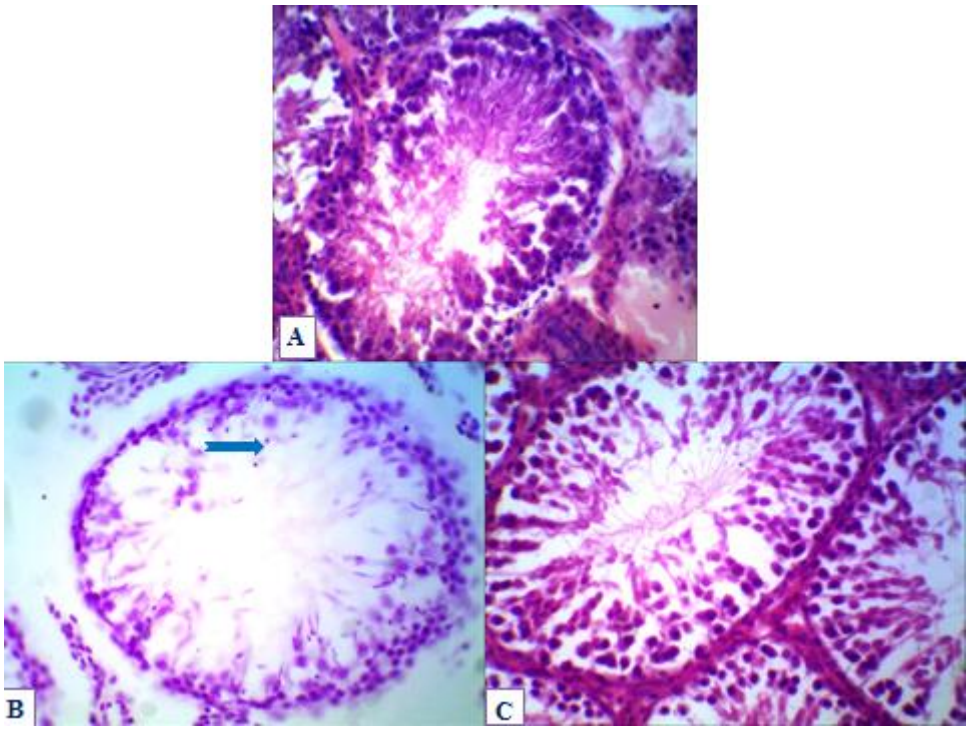

Figure 3 A) Histological light photomicrograph of testis sections (H and E400×) of control group shows normal arrangement of seminiferous tubules, sperms and normal architecture interstitials cells. B) A section from untreated diabetic rat shows disorganization of spermatogenic cells, degeneration of germinal epithelium, and multinucleated cells and destroyed epithelium into the lumen Seminiferous tubules have a slight loss of epitheliumand improved spermatogenesis in GT + DM group is seen. karyolysis (blue arrow) are seen. C) Light photomicrograph of green tea treated section shows the seminiferous tubules have partially recoverd to their normal structure.

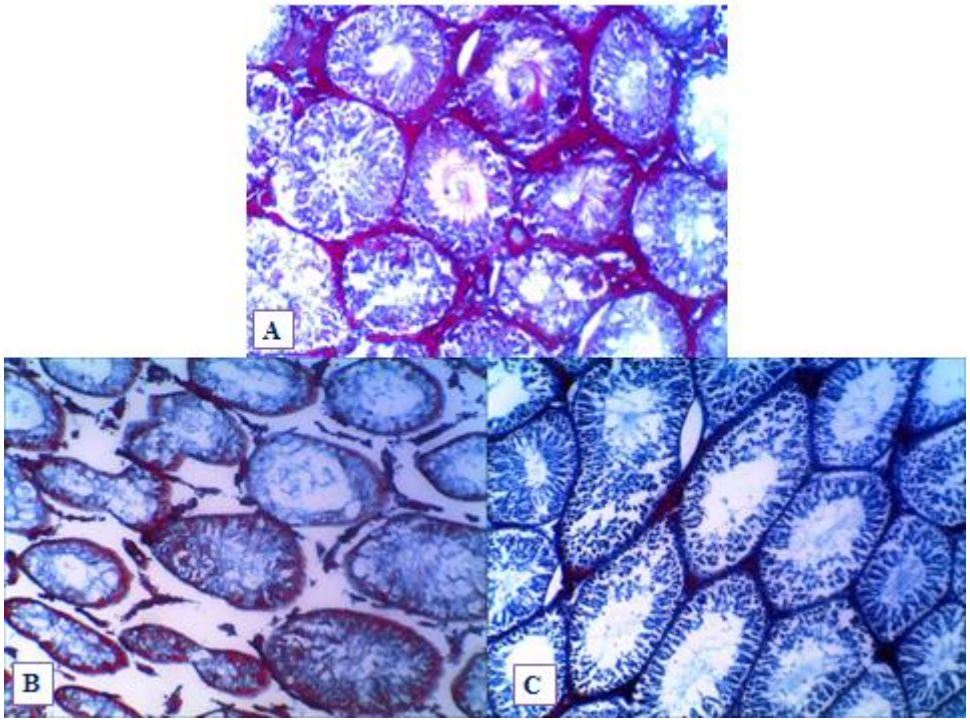

Figure 4 A) Normal collagen fibers distribution in the control group of testis sections B) A section form group II shows rise in the diameter of the interstitial spaces and noticable reduction of collagen fibers C) a section in group III treated with green tea shows light collagen fiber distribution (Mallory's trichrome 200×).

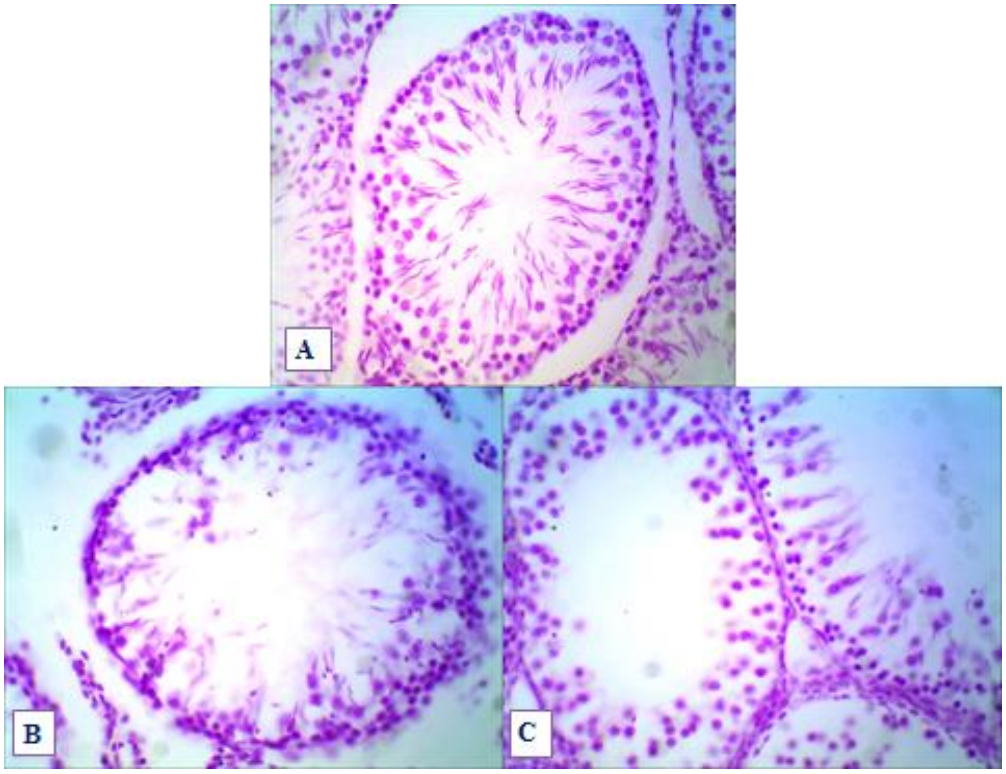

Figure 5 A) Normal organization of germ cells of the control group B) A disorganization and depletion of the germinal epithelium in the STs. indiabetic rat C) The germinal epithelium recoverd to its normal structure in the section of green tea treated rats of group III (Feulgen stain 400×)

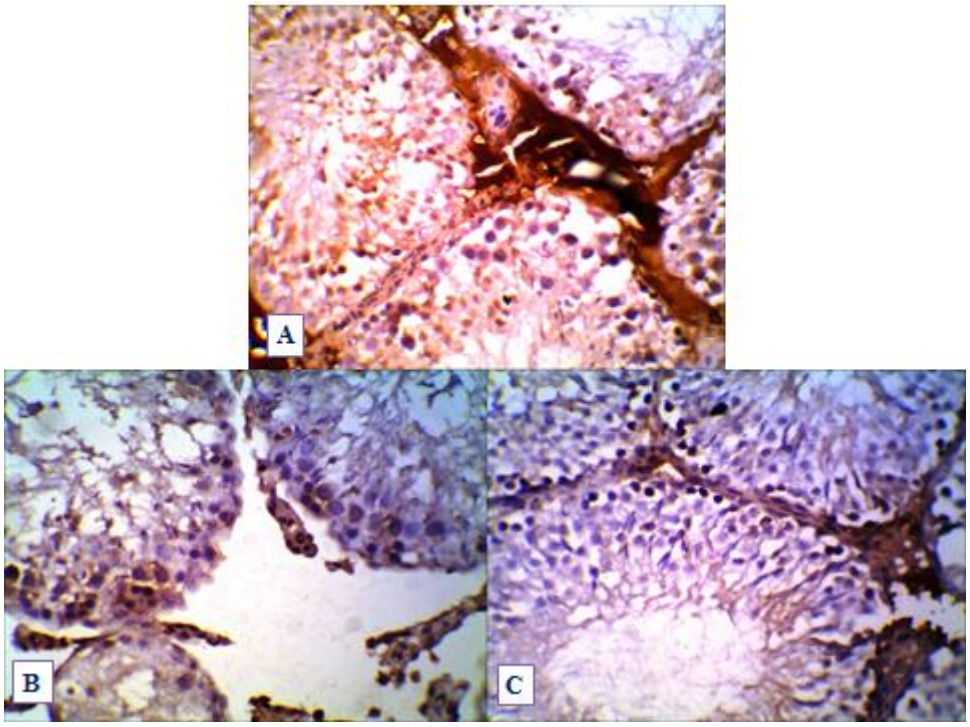

Figure 6 A) Moderate expression of Caspase-3 immunostaining of germinal cells of control group. B) Marked expression of Caspase-3 of diabetic group. C) Also, marked expression of Caspase-3 in the S.Ts germinal cells of green tea treated rats of group III (Caspase-3 immunostaining 400×)

Table 1 The seminiferous tubules diameters and thickness as well as collagen fibres percentage. The number of the caspase-3 +ve for the different groups explained as mean ± SD.

Study groups	Average thickness of the S.T.	Average diameters of S.T.	Average area percentage of the collagen fibers S.T. tubular	Average number of the caspase-3+ve cells of the S.T. germinal cells
Control (Group I)	94.86± 6.57	177.12± 14.92	152.03± 23.25	12.00± 1.84
Diabetic (Group II)	180.70± 21.40	126.63± 32.18	65.87± 15.85	34.71± 2.87
Green tea treated (GroupIII)	66.65± 11.07	159.13± 19.99	67.93.11± 10.72	32.82± 2.65

3. DISCUSSION

Our study results showed that green tea has considerable protective effects against morphological changes of testicular tissues. The antioxidant properties of green tea may be responsible for this protection. It is possible that free radicals are caused by tissue injury associated with diabetes and its subsequent complications (Lapolla et al., 2005). Tea is a frequently consumed drink world wide, especially green tea, which contains phenolic compounds including the epigallo catechin gallate, catechins, epicatechin gallate. The antioxidant activity of green tea polyphenols and the pro-oxidant effects of these compounds, leading to indirect antioxidant effects, have also been suggested as potential mechanisms for prevention of cancer (Barghout et al., 2020; Hou et al., 2005; Butt & Sultan, 2009).

Another study showed, green tea extract significantly increased levels ofserum insulin in the diabetic rats, and green tea extract (combined with ginseng roots) protected the cells in the islets of Langerhans (Karaca et al., 2010). Other studies have indicated increased thickness of S.Ts and germ cell depletion human with diabetes and also diabetic rats (Cameron et al., 1985; Sadik et al., 2011). Various herbal extracts or are useful for treatment of diabetes and other metabolic diorders (Samad et al., 2009). Accordingly, antioxidant medication is a main plan for treating diabetes (Samad et al., 2009). Antioxidant enzymes eliminate harmful reactive oxygen species in male reproductive organs. It also playing a vital role in preserving reproductive functions (Fujii et al., 2003).

Green tea extract helps to improve diabetic renal changes mainly by correcting oxidative stress as shown in another previous study (Pedraza-Chaverrí et al., 2004). There are many studies that support on the benefits of antioxidants in protecting the testis from oxidative stress (Abdallah et al., 2019; El-Sokkary et al., 1999; Lombardo et al., 2011; Maneesh et al., 2005).

5. CONCLUSION

The green tea has antioxidant activities and reduces the side effects of diabetes mellitus on testicular structures. A siginificant improvements was revealed in the morphological changes of testicular tissues observed in diabetic groups after treatment green tea.

Acknowledgments

This publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University, Alkharj, Saudi Arabia. In addition we thank those who participated and contributed to the study.

Authors' Contributions

All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A. Ali and Ali Yaqoub Alali participated in the study design and wrote the first draft of the manuscript. Rasheed A. Alhajri, Abdulrahman M. Aldawsari, and Ammar H. Al Heseni collected and processed the samples. Nasser S. Aldosari participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

Funding

This study has not received any external funding.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

The data are available upon request from the authors.

Ethics Approval

All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (PSAU-2020 ANT 1/39PI).

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Abdallah HM, Abdel-Rahman RF, El Awdan SA, Allam RM, El-Mosallamy AE, Selim MS, Mohamed SS, Arbid MS, Farrag AR. Protective effect of some natural products against chemotherapy-induced toxicity in rats. Heliyon. 2019; 5(5):e01590.
2. Adel AG, Sabah EF, Tarek S, El-Naggar A. Protective role of green tea on carbon tetrachloride-induced acute hepatotoxicity. Sci J Fac of Sci. (Minufiya University) 2009; 23:55-67.
3. Al-Attar AM, Abu Zeid IM. Effect of tea (Camellia sinensis) and olive (Olea europaea L.) leaves extracts on male mice exposed to diazinon. Bio Med Res Int 2013; 2013.
4. Bagherpour H, Malekshah AK, Amiri FT, Azadbakht M. Protective effect of green tea extract on the deltamethrin-induced toxicity in mice testis: An experimental study. Int J Reprod Bio Med 2019; 17(5):337.
5. Ballester J, Muñoz MC, Domínguez J, Rigau T, Guinovart JJ, Rodríguez-Gil JE. Insulin-dependent diabetes affects testicular function by FSH-and LH-linked mechanisms. J Androl 2004; 25(5):706-19.
6. Bancroft JD, Gamble M. Theory and practice of histology techniques. Churchill Livingstone Elsevier, London. 2008; p83-134
7. Barghout B, El-Sayed S, El-Said ES. Protective effect of green tea extract against cadmium-induced testicular damage in rats in respect of oxidant/antioxidant equilibrium and androgen production. Man Vet Med Jour 2020; 21(1):31-5.

 ISSN 2321–7359 EISSN 2321–7367 I OPEN ACCESS

8. Butt MS, Sultan MT. Green tea: nature's defense against malignancies. Cri rev food sci nutrition 2009; 49(5):463-73.
9. Cai L, Chen S, Evans T, Deng DX, Mukherjee K, Chakrabarti S. Apoptotic germ-cell death and testicular damage in experimental diabetes: prevention by endothelin antagonism. Urol Res 2000; 28(5):342-7.
10. Cameron DF, Murray FT, Drylie DD. Interstitial compartment pathology and spermatogenic disruption in testes from impotent diabetic men. Anat Rec 1985; 213(1):53-62.
11. El-Sokkary GH, Reiter RJ, Tan DX, Kim SJ, Cabrera J. Inhibitory effect of melatonin on products of lipid peroxidation resulting from chronic ethanol administration. Alcohol 1999; 34(6):842-50.
12. Elzoghby RR, Ahlam FH, Abdel-Fatah A. Protective role of vitamin C and green tea extract on malathion-induced hepatotoxicity and nephrotoxicity in rats. Am J Phar Toxic 2014; 9:177.
13. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress− activated signaling pathways mediators of insulin resistance and β-cell dysfunction?. Diabetes 2003; 52(1):1-8.
14. Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr 2003; 133:3275S-84S.
15. Fujii J, Iuchi Y, Matsuki S, Ishii T. Cooperative function of antioxidant and redox systems against oxidative stress in male reproductive tissues. Asian J Androl 2003; 5(3):231-42.
16. Hamden K, Carreau S, Ellouz F, Masmoudi H, Feki EI. Improvement effect of green tea on hepatic dysfunction, lipid peroxidation and antioxidant defence depletion induced by cadmium. Afr J Biot 2009; 8(17).
17. Hassen MT, Mohamed HK, Montaser MM, El-Sharnouby ME, Awad N, Ebiya RA. Molecular, Immunomodulatory, and Histopathological Role of Mesenchymal Stem Cells and Beetroot Extract on Cisplatin Induced Testicular Damage in Albino Rats. Animals 2021; 11(4):1142.
18. Heikal TM, Mossa AT, Rasoul MA, MAREI GI. The ameliorating effects of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats. changes 2013; 5(9).
19. Higdon JV, Frei B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr 2003; 43:89-143.
20. Hou Z, Sang S, You H, Lee MJ, Hong J, Chin KV, Yang CS. Mechanism of action of (−)-epigallocatechin-3-gallate: Auto-oxidation–dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. Cancer Res 2005; 65(17):8049-56.
21. Karaca T, Yoruk M, Yoruk IH, Uslu S. Effects of extract of green tea and ginseng on pancreatic beta cells and levels of serum glucose, insulin, cholesterol and triglycerides in rats with experimentally streptozotocin-induced diabetes: a histochemical and immunohistochemical study. J of An and Vet Advan 2010; 9(1):102-7.
22. Kesavulu MM, Giri R, Rao K, Apparao CH. Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. Diabetes Metab 2000; 26(5):387-92.
23. Kuhn-Velten, N., Waldenburger D., Staib, W. Evaluation of Steroid Biosynthetic Lesions in Isolated Leydig Cells from the Testes of Streptozotocin-Diabetic Rats. Diabetologia 1982; 23: 529-533.
24. Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, Tsubono Y, Tsuji I. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. JAMA 2006; 296(10):1255-65.
25. Lapolla A, Fedele D, Traldi P. Glyco-oxidation in diabetes and related diseases. Clin Chim Acta 2005; 357(2):236-50.
26. Lombardo F, Sansone A, Romanelli F, Paoli D, Gandini L, Lenzi A. The role of antioxidant therapy in the treatment of male infertility: an overview. Asian J Androl 2011; 13(5):690 7.
27. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Bio Med 2006; 41:1727-46.
28. Malaisse WJ. Alloxan toxicity to the pancreatic B-cell: A new hypothesis. Bio Med Pharmacol 1982; 31(22):3527-34.
29. Maneesh M, Jayalakshmi H, Dutta S, Chakrabarti A, Vasudevan DM. Experimental therapeutic intervention with ascorbic acid in ethanol induced testicular injuries in rats. Indian J Exp Biol 2005; 43(2):172-6.
30. Moore H, Summerbell C, Hooper L, Cruickshank K, Vyas A, Johnstone P, Ashton V, Kopelman P. Dietary advice for treatment of type 2 diabetes mellitus in adults. The Cochrane database Syst Rev 2004; 2: 43-50.
31. Morimoto, S., Mendoza-Rodriguez, A., Hiriat, M. Protective effect of testosterone on early apoptotic damage induced by streptozotocin in rat pancreas. J Endocrinol 2005; 187: 217-224.
32. Oztürk F, Gül M, Agkadir M, Yagmurca M. Histological alterations of rat testes in experimental diabetes. T Kin J Med Sci 2002; 22:173-8.
33. Pedraza-Chaverrí J, Barrera D, Maldonado PD, Chirino YI, Macías-Ruvalcaba NA, Medina-Campos ON, Castro L, Salcedo MI, Hernández-Pando R. S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative

stress and renal damage in vivo. BMC Clin Pharmacol 2004; 4(1):1-3.

34. Sadik NA, El-Seweidy MM, Shaker OG. The antiapoptotic effects of sulphurous mineral water and sodium hydrosulphideon diabetic rat testes. Cell Physiol Biochem 2011; 28(5):887-98.
35. Samad A, Shams MS, Ullah Z, Wais M, Nazish I, Sultana Y, Aqil M. Status of herbal medicines in the treatment of diabetes: a review. Curr Diabetes Rev 2009; 5(2):102-11.
36. Sanguinetti RE, Ogawa K, Kurohmaru M, Hayashi Y. Ultrastructural changes in mouse Leydig cells after streptozotocin administration. Exp Anim 1995; 44(1):71-3.
37. Sebastian R, Raghavan SC. Endosulfan induces male infertility. Cell Death Dis 2015; 6: e2022.
38. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of Gongronema latifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J Biosci 2003; 28(1):1-5.
39. Vieira JI, Silva TA, Barbosa WM, Azevêdo GL, Arruda LC, Guerra MM, Soares PC, Silva EC. Effect of green tea extract (Camellia sinensis) on the spermatic parameters of Wistar rats submitted or not to testicular heat shock. An Reprod 2020; 17(2).
40. Wazaify M, Afifi FU, El-Khateeb M, Ajlouni K. Complementary and alternative medicine use among Jordanian patients with diabetes. Complement Ther Clin Pract 2011; 17(2):71-5.